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# A critical comparison of cell-based sensor systems for the detection of Cr(VI) in aquatic environment

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ABSTRACT

The toxicity of chromium ions was investigated using mammalian cell cultures on impedance sensors as well as physiological in vitro sensor systems. The performance of commercially available systems like the 2500 Analyzing System (Bionas), xCELLigence (Roche) and Cytosensor Microphysiometer (Molecular Devices) was compared with a novel CMOS-based impedance-to-frequency converter device. Cell-based sensor systems are shown to be powerful tools to detect Cr(VI) pollutions within several hours in the range of multinational drinking water regulations. The ability to distinguish between toxic Cr(VI) and non-toxic Cr(III) species is one advantage of these integral sensor systems. Impedance only devices are not sufficient for the fast detection of toxic chromium species as rapid cellular changes occur only in the respiration system and the cell physiology.



## 1. Introduction

Chromium (Cr) is a metal of widespread use which is present in the environment in two major stable oxidation states: Cr(III) and Cr(VI). Cr(III) occurs naturally and is a nutritional supplement that helps insulin to regulate the sugar levels in the human body. An intake of 50–200 mg of Cr(III) per day is recommended for adults [1]. Cr(III) forms octahedral coordination compounds, complexes and chelates that usually cannot cross cell membranes and therefore the toxicity is considered to be relatively low [2]. On the other side highly water soluble Cr(VI) at and above physiological pH (>6), exists as a chromate oxyanion $(CrO_4)^{-2}$ and can readily cross cell membranes through the sulphate anion transport system [3]. Cr(VI) compounds have been shown to be 1000-fold more potent than Cr(III) compounds in inducing cytotoxicity and mutagenesis in the same cell type [4,5]. Extensive use of chromium in many industries such as electroplating, steel productions, pigment manufacturing, wood preservation and leather tanning results in releasing chromium containing effluents to the environment making it a non-negligible threat to the ecological system. Different studies indicate the integration of multiple pathways involved in Cr(VI) toxicity but the exact mechanisms is still unclear. The anionic transport system, along with intracellular reduction reactions, allows chromium to accumulate in cells at concentrations much higher than the extracellular levels [6]. Inside the cell, it is reduced to reactive intermediates, such as Cr(V), Cr(IV) and Cr(III) by reducing enzymes and cellular reductants [7,8]. Inside the cell, Cr(III) reacts with cellular macromolecules like DNA and intracellular reducers like GSH or cysteine [9]. During its reduction reactive oxygen species are produced which are thought to be responsible for many of the toxic effects caused by Cr(VI). The high toxicity has been recognized on the basis of epidemiological evidence and therefore, European Commission has set the maximum level of total chromium allowed in drinking water at 50 μg/l [10].

Standard analytical chemical methods are used for the detection of chromium in water samples, like ion chromatography [11] or atomic-absorption spectrometry [12]. Beside these classical methods, different biosensors for environmental application, capable of detecting Cr(VI), have been developed too, like amperometric enzyme-based sensors using cytochrome $c_3$ [13] or

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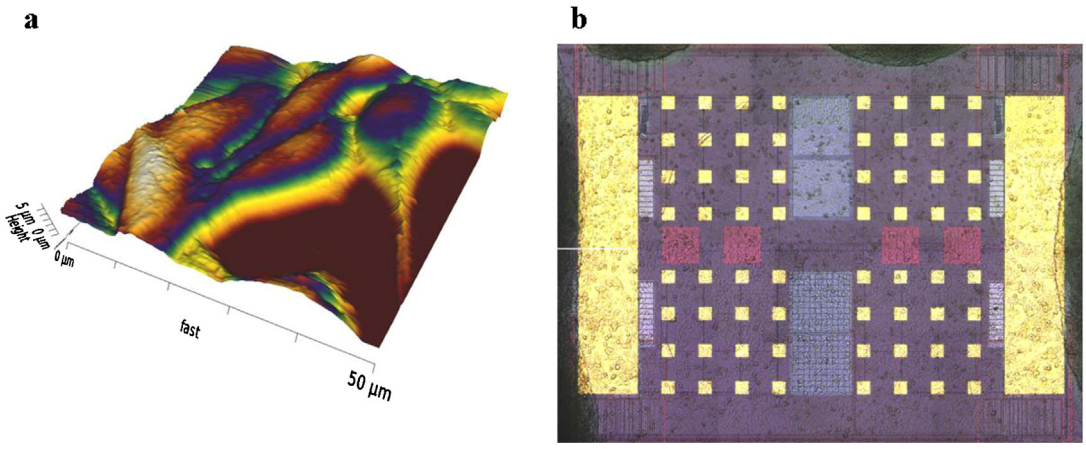


**Fig. 1.** (a) Atomic force microscopy (AFM) image of V79 hamster lung fibroblast cells on the surface of an untreated glass slide (fixed with 1.25% glutaraldehyde; 25 min). V79 cells form a confluent monolayer. (b) Light microscopy image of living V79 cells on a CMOS chip.

spectrophotometric enzyme inhibition assays [14], but also cell-based sensors using bacteria [15].

Living cells are continuously integrating different sources of chemical and physical signals originating from both internal and external environments [16]. The use of cell-based sensors has increased in the last few years [17]. Important areas of research are the toxicity monitoring in aquatic environments [18,19] and also the monitoring of toxic gaseous compounds in the air, which came into the focus of cell-based sensor research [20]. One of the main reasons for the recent success is the development of cell-based sensor systems which can be used in field applications [21]. In this context, Electrical cell impedance spectroscopy [22] has been demonstrated to be feasible to monitor various aquatic toxicants including heavy metals and industrial pollutants [23]. Cr(VI) detection using cell-based impedance sensors was already performed before, using the Real-Time Cell Electronic Sensor [24]. Generally, heavy metals are the most insidious pollutants because of their non-biodegradable nature and ability to persist for long periods [25]. The market for cell-based sensor systems is growing and led to commercially available devices (Table S1) [26,27].

The aim of this study was to test and compare four cell-based biosensors for the detection of Cr(VI) pollution. The following methods were used in this study for the detection of Cr(VI) and Cr(III) in aqueous solutions: 2500 Analyzing System (Bionas), xCELLigence (Roche), Cytosensor Microphysiometer (Molecular Devices), CMOS chip (complementary metal oxide semiconductor; in house). The sensitivity of commercially devices as well as novel cell-based sensor systems was evaluated and compared to standard cell viability assays.

## 2. Materials

### 2.1. Chemicals and cell culture

$K_2Cr_2O_7$, $CrCl_3$ and Triton X-100 were purchased from Sigma-Aldrich (Steinheim, Germany). The Chinese hamster lung fibroblasts cells (V79) (DSMZ, Braunschweig, Germany) were cultured at 37 °C and 5% $CO_2$ in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Darmstadt, Germany) which was supplemented with 10% fetal bovine serum (FBS) (Biochrom, Berlin) and penicillin 100 Units/ml, and streptomycin (100 μg/ml). This fast growing cell line (doubling time: 9–12 h) forms confluent cell monolayers within short time (Fig. 1a). Cells were subcultured at 90% confluency, and cells within passage 5–20 were used in the experiments.

## 3. Methods

### 3.1. CMOS-Chip

A novel CMOS-based impedance chip was used for this study (Fig. 1b) [28]. The working principle is described elsewhere [29]. In brief, the impedance is measured on 64 gold microelectrodes (area $3 \times 10^{-5}$ $cm^2$) by an integrated circuit on the chip. The measured impedance values are encoded in the form of the frequency of the digital output signal which is proportional to the impedance. The sensor front-end electronics are integrated in close physical proximity to the sensing site to keep the sensor interface to the external world as simple and robust as possible.

V79 cells ($1 \times 10^6$) in 2 ml full growth DMEM (10% FBS) were seeded on the sensor chip. During the measurement, the chip and support printed circuit board were placed in a humidified incubator (5% $CO_2$, 37 °C) to maintain appropriate culture conditions. 20–21 h after the cells were cultured on the sensor, cells were exposed to 50 μM Cr(VI) solution in DMEM. The addition of chromium has no effect on the cell culture medium pH, which was controlled in the medium with the highest concentration ($\Delta$pH < 0.1). The impedance was recorded every 60 s to monitor the response of the cells to the Cr(VI) treatment.

### 3.2. Roche xCELLigence

The Roche xCELLigence SP device which has been used in this study, is described in detail elsewhere [26]. The technology was already used in previous studies for the investigation of heavy metals including chromium [30]. In brief, it consists of an electronic sensor analyzer, a device station and a 96-well plate. The gold electrode sensors on the bottom of the wells are designed to cover about 80% of the bottom surface. The device station was capable of electronically switching any one of the wells to the sensor analyzer for impedance measurement. In operation, the device station was placed inside an incubator. Electrical cables connect the device station to the sensor analyzer. Measurements on the wells are continuously conducted. To represent cell status based on the measured electrical impedance, the cell index (CI) is calculated by

$$\mathrm{CI} = \max_{i=1,\ldots,N} \left[ \frac{R_{\mathrm{cell}}(f_i)}{R_b(f_i)} - 1 \right]$$

where $N$ is the number of the frequency points at which the impedance is measured [31]. At the beginning of an experiment, when no cells are present on the electrodes or when the cells are not well-attached onto the electrodes, $R_{\mathrm{cell}}(f)$ is the same as $R_b(f)$

(background impedance of the medium), leading to CI = 0. If more cells attach onto the electrodes, a larger impedance value will be detected leading to a increased CI value. Thus, CI is a quantitative measure of the number of cells attached to the sensors. For the same number of cells attached to the sensors, cell death or toxicity induced cell detachment or cells rounding up will lead to a smaller CI [30]. V79 cells are loaded into each well of the sensor devices (40,000/well) at the start of each experiment to allow attachment on the sensor. The 96-well plate with cells was then mounted back to the device stations placed inside a humidified incubator (37 °C; 5% $CO_2$). After 21 h, the growth medium was replaced by chromium containing DMEM and the sensor plate was incubated again for 22 h. The CI was monitored by the xCELLigence system every 60 min. For this setting, full confluency was not achieved at the end of the experiments.

### 3.3. Molecular devices cytosensor microphysiometer

The Cytosensor, based on the light-addressable potentiometric sensor technology (LAPS), is one of the oldest commercial cell-based sensor systems [32,33]. Research groups worldwide have made attempts to improve the LAPS based sensors system technology [34–37]. The Cytosensor was used in the analysis of membrane bound receptors [38] and non-receptor mediated events on cell metabolism like the detection of heavy metals [39].

Cells are cultured on polycarbonate membranes and placed in the sensor chamber in vicinity to the sensor chip surface which is able to detect changes in the pH. The cells are fed with medium in a stop-go mode (20 s stop-phase – 100 s go-phase). The sensor itself consists of a silicon chip coated with a thin insulating layer of silicon oxynitride. During hydration, the insulating layer reacts with the aqueous solution resulting in silanol (Si–OH) and silamine ($Si–NH_2$) groups which are affected by the pH of the solution. An infrared light from an LED at the backside of the chip produces a photocurrent which depends on the sum of the applied and the surface potential. As the photocurrent is kept constant by adjusting the voltage, changes in the applied voltage indicate changes in the pH. Voltage changes over time can be plotted as 'raw data'. Plotting the negative slope of the 'raw data' against the time results in 'raw rates'. The basal acidification rate is set to 100% and the cellular response is given in percentage over basal acidification.

### 3.4. Bionas 2500 Analyzing System

Investigations of cytotoxic effects of various kinds of heavy metals have been performed using a Bionas 2500 Sensor chip system [40]. The sensor chip enables continuous measurement of oxygen consumption using Clark-type electrodes, pH changes of the medium by employing ion-sensitive field effect transistors and the impedance between two interdigitated electrode structures [41,42] to measure the impedance under and across the cell layer on the chip surface. Before measurement, cells were seeded on the sensor chip (SC 1000 Metabolic Chip) and incubated until confluency was reached. Sensor chips with cells were then transferred to the 2500 Analyzing System in which medium was exchanged in 3 min cycles (3 min pump-go–3 min pump-stop) during which the parameters were measured. The running medium (RM) during the analysis was weakly buffered (1 mM Hepes) DMEM with reduced FBS (1%). For toxicity testing, the following steps were conducted: (a) 5 h equilibration with running medium with 3 min flow/stop incubation intervals, (b) 36 h Cr incubation with substances freshly added in medium at indicated concentrations with the same flow/stop cycles, (c) removing of cells by addition of 0.2% Triton X-100 to obtain a basic signal without living cells on the sensor surface as a negative control.

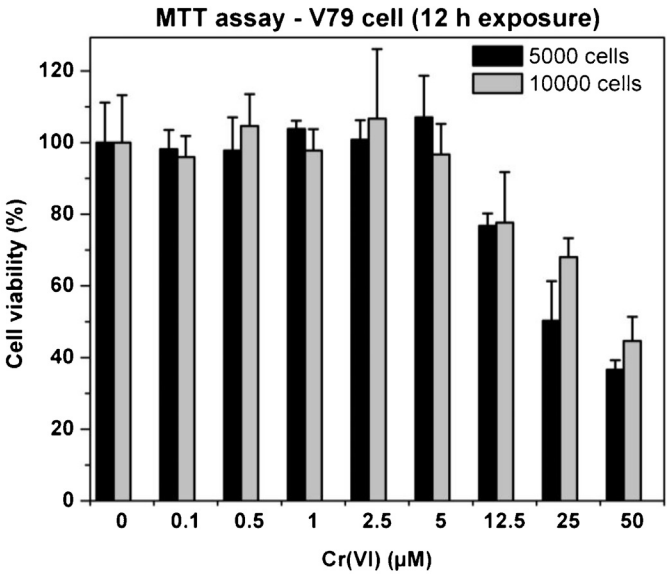


**Fig. 2.** Cell viability of V79 cells (5000 and 10,000) assessed by MTT assay after exposure to different concentrations of Cr(VI) (0–50 μM) for 12 h. The results were reported as mean ± SD of three independent experiments.

### 3.5. Cell viability assay – MTT assay

Cell viability after exposure to different chromium concentrations was assessed by measuring reduction of the tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] by mitochondrial succinate dehydrogenase [43]. The MTT cell proliferation assay kit I (Roche Diagnostics, Mannheim, Germany) was used following manufacturer's instructions. V79 cells (5000 and 10,000) were seeded into each well of a 96-well plate (Greiner, Frickenhausen, Germany) and incubated over night. After the attachment, the medium was replaced with chromium solutions, resulting in a volume of 90 μl per well. Negative controls (DMEM) and positive controls (1% Triton X-100) were also included in every experiment. After 12 h incubation, 10 μl of MTT solution was added to each well (final concentration 0.5 mg/ml). Incubation was terminated after 4 h by adding 100 μl of solubilization solution to each well. The plates were incubated until the formazan salts were dissolved. Finally, the absorption was measured at 595 nm (test wavelength) and 640 nm (reference wavelength) using a FLUOstar microplate reader (BMG Labtechnologies, Offenburg, Germany).

## 4. Results and discussion

### 4.1. Cell viability of V79 after 12 h Cr(VI) exposure

MTT assay experiments have been performed with V79 cells. Cells were exposed to Cr(VI) at concentrations from 0–50 μM Cr(VI) (Fig. 2). Low Cr(VI) concentrations of up to 5 μM showed no adverse effects compared with the control measurement. A cytotoxic reaction is first observed at 12.5 μM Cr(VI). With increasing Cr(VI) amounts, the cell viability decreases continuously. This effect is more pronounced in experiments with the lower cell density of 5000 cells, which is in accordance to other groups presenting reduced fibroblast growth and cell division after exposure to Cr(VI) in V79 cells [44]. At lower cell densities, the amount of cells is not sufficient to build a confluent monolayer which acts slightly as a diffusion barrier for Cr(VI), leading to a decreased individual cell surface exposed to the polluted medium. In addition, at high cell densities, the cells have more surrounding space and tend

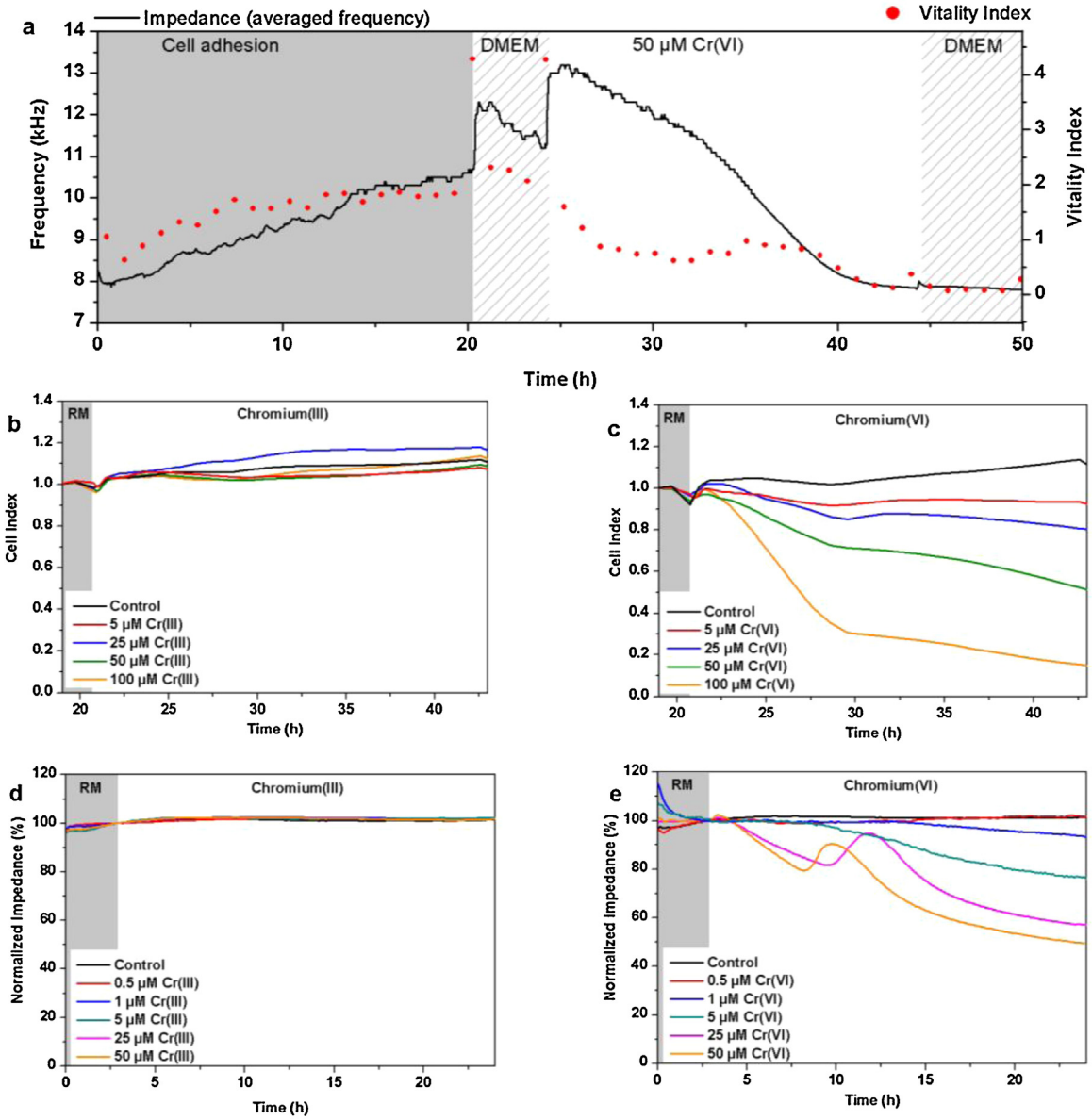


**Fig. 3.** Time-transient of the impedance measurements of V79 Chinese hamster lung cells exposed to different chromium species. (a) 50 µM Cr(VI) solution monitored with an in-house impedance-to-frequency CMOS chip; different concentrations of (b) non-toxic Cr(III) and (c) toxic Cr(VI) solutions monitored with an xCELLigence SP device; different concentrations of (d) non-toxic Cr(III) and (e) toxic Cr(VI) solutions monitored with a 2500 Analyzing System. Prior to every measurement, an adaption phase with pure medium was added (gray shadow).

to divide. However, this might corresponds to an elevated cellular metabolism which increases the uptake of the Cr(VI).

### 4.2. Impedance measurements of Cr treated cells

#### 4.2.1. Impedance measurements with CMOS-chip

The CMOS-chip records individual curves for each of the 64 sensor electrodes. Previous experiments [29] showed that not only the impedance value itself but also the amount of fluctuation in the signals from individual electrodes is an indicator of the cells' vitality and mobility. For an easier and more consistent interpretation of this parameter, it should be quantified in a single number. A cell vitality index is therefore introduced, which is calculated as follows: The chip frequency data is segmented into blocks of $N$ data points (here $N = 40$, corresponding to approximately 1 h of the experiment). Changes of the electrode signals indicate the presence of moving and therefore vital cells which causes variations in the coverage of the electrodes. Higher variations are correlated to an increased micromotion of the cells. Within each block, the sum of the relative standard deviations of all electrodes is defines as the overall cell vitality index for this block. The usefulness of this calculated number is demonstrated by the results of the conducted chromium measurements. Fig. 3a shows the output from the integrated impedance sensor as both the average frequency of all electrodes (black line) and the cell vitality index (red circles). In the beginning, the V79 cells on the chip are allowed to grow for about 21 h. In this time, both the mean output frequency of the circuit and the vitality index rise as the cells spread out and attach to the chip surface, forming a confluent monolayer (Fig. 1b). The vitality index already stabilizes at a value around 2 after 10 h, while the absolute frequency continues to rise. The used medium was then exchanged for 2 ml of fresh DMEM, which causes a rise of the average frequency [28,29]. The vitality index does not increase as much except one outlier which is a numerical artifact caused by the sudden jump of the absolute frequencies.

After four more hours, the medium on the chip was again exchanged, and 2 ml DMEM at a concentration of 50 μM Cr(VI) was added onto the chip. In response, the average frequency first increases, and then starts to fall. The cell vitality index immediately drops to values as low as at the beginning of the experiment, indicating a drastic reduction of the cells' micromotion. In the following hours, the average frequency also falls drastically presumably because the cells have become lethally damaged. After 20 h of exposure to Cr(VI), the medium was replaced with DMEM without Cr(VI) again in order to ascertain whether the effect could be reversed. The experiment was continued for additional 24 h (data not shown), but the signals remained stagnant after the end of the experiment, which showed that the cells were rounded and only loosely resting on the chip surface.

These very first results of this novel CMOS impedance sensor demonstrate that evaluating both the absolute values and the variability (fluctuations) of the measured impedance data helps to obtain a more complete picture of the health of the cultured cells.

#### 4.2.2. Impedance measurements with Roche xCELLigence

The impedance measurements, performed with the xCELLigence system, show a clear distinction between Cr(III) and Cr(VI). While Cr(III) did not alter the impedance results, even at concentrations of 100 μM (Fig. 3b), Cr(VI) leads to a concentration dependent decrease of the impedance (Fig. 3c), displayed by the CI. Within the first 10 h, a steeper decrease of the CI values is observed compared to the following minor decrease, which might indicate the change in the cellular response toward the Cr(VI) exposure. Concentrations down to 5 μM Cr(VI) could be detected after 24 h of exposure. Compared to former impedance studies performed with NIH-3T3 cells and a RT-CES system, the V79 cells are less sensitive within the same exposure time [30].

#### 4.2.3. Impedance measurement with Bionas 2500 Analyzing System

The impedance measurements indicate the breakdown of the cytoskeleton. Cr(III) did not affect the cellular impedance (Fig. 3d). At Cr(VI) concentrations higher than 5 μM, a reduction of the impedance is observable (Fig. 3e). Within 5 h, the reduction is larger than 10% of the control measurement. The impedance values re-increase after 5–7 h for the concentrations of 25–50 μM. The increase lasts for approximately 2 h before the impedance falls again constantly. A biphasic decrease of the impedance, as shown above in Fig. 3d with the xCELLigence system, was not observed, although the sensitivity of these devices was comparable for the chosen combination of cell culture and test substance. Detection limit for Cr(VI) detection is 5 μM, as lower concentration of 1 and 0.5 μM Cr(VI) are within more than 90% of the control measurement and therefore hardly separable.

### 4.3. Measurement of acidification rates

#### 4.3.1. Cytosensor microphysiometer

Fig. 4a shows the acidification of unaffected V79 cells which were incubated over the whole time with Hepes buffered running medium (black curve, control). The linear increase is only altered directly after the medium change which might be due to differences in the temperature. The non-toxic Cr(III) solution does not affect the acidification rate as the red curve (50 μM Cr(III)) is more or less congruent with the control curve (Fig. 4a). In contrast, toxic Cr(VI) solutions lead to a clear reduction of the acidification rate over an extended time period of 5 h. In the first 2 h of the Cr(VI) incubation an increase of the acidification rate is observable which might be the result of an activation of the glycolysis to compensate the loss in energy production of the inhibited respiratory pathways. The decrease of the acidification rates is concentration dependent leading to the signal reduction down to 60% after 5 h of incubation with 150 μM Cr(VI) solution.

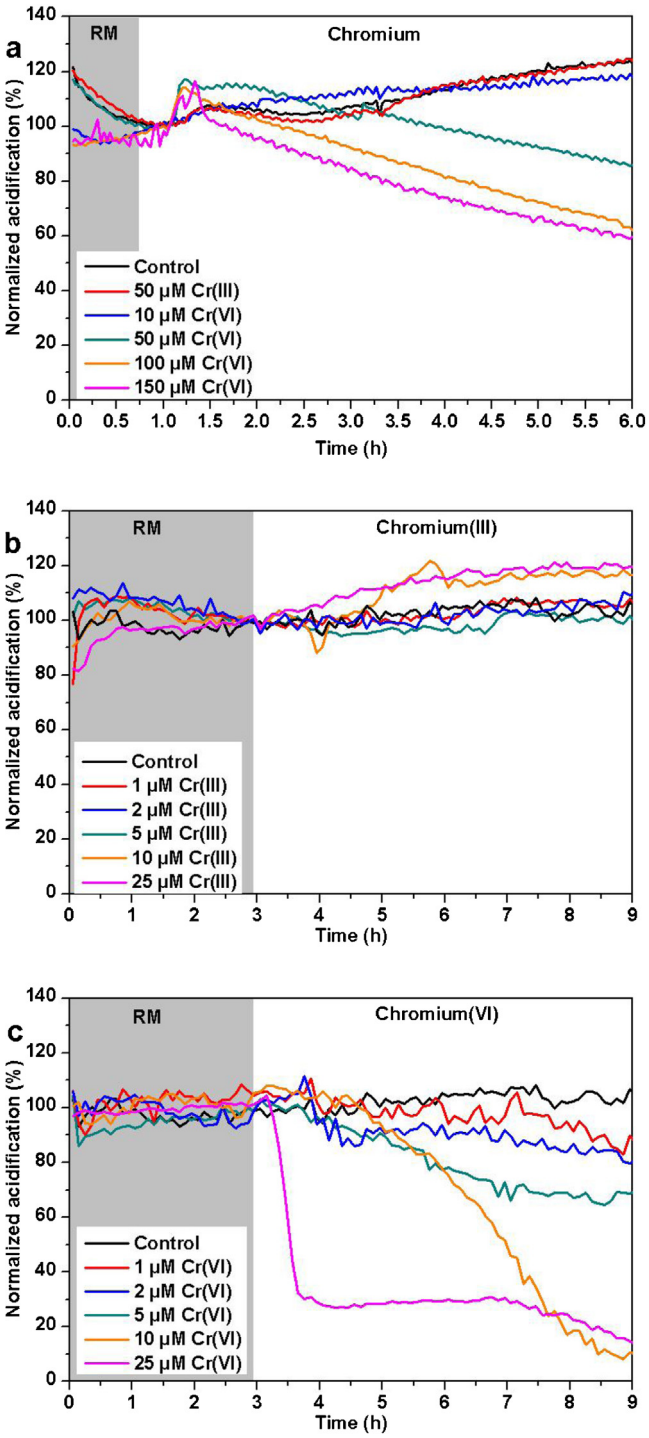


**Fig. 4.** (a) Time-transient of normalized acidification rate measurements of V79 Chinese hamster lung cells incubated with different concentrations of non-toxic Cr(III) and toxic Cr(VI) solution for 5 h, monitored with a Cytosensor Microphysiometer. Time-transient of normalized acidification rate measurements of V79 Chinese hamster lung cells incubated with different concentrations of (b) non-toxic Cr(III) and (c) toxic Cr(VI) solution for 5 h, monitored with a 2500 Analyzing System. Prior to every measurement, an adaption phase with pure medium was added (gray shadow).

#### 4.3.2. Bionas 2500 Analyzing System

The extracellular acidification rates reflect in a simplified manner the metabolism of the cells, especially the glycolytic activity.

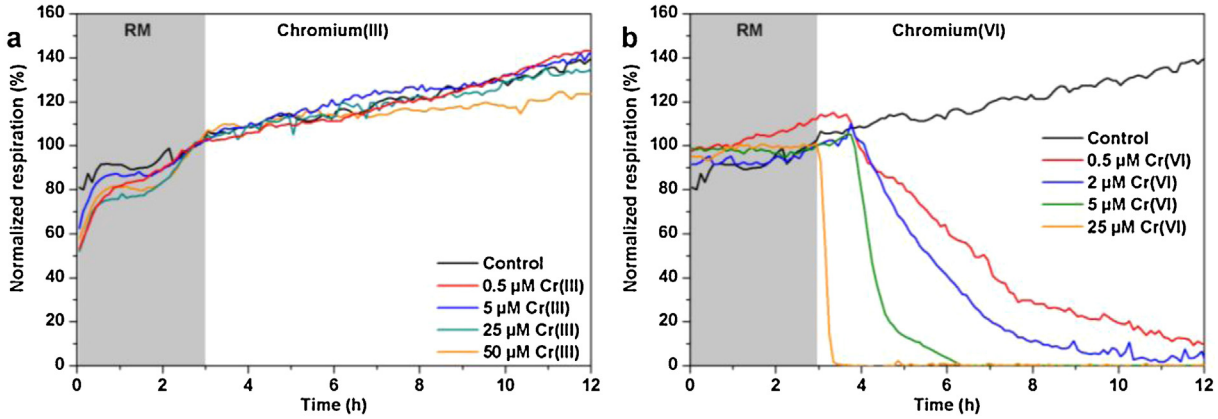


**Fig. 5.** Time-transient of normalized respiration rate measurements of V79 Chinese hamster lung cells incubated with different concentrations of (a) non-toxic Cr(III) and (b) toxic Cr(VI) solution for 5 h, monitored with a 2500 Analyzing System. Prior to every measurement, an adaption phase with pure medium was added (gray shadow).

Cr(III) is hardly influencing the cellular metabolism (Fig. 4b), which is similar to the revealed results of the Cytosensor Microphysiometer in Fig. 4a. Cells exposed to Cr(VI) showed a decrease in the acidification rates, measured with the 2500 Analyzing System, within the first 6 h of treatment (Fig. 4c). Within the first 3 h of Cr(VI) exposure, chromium concentrations down to 5 μM cause an acidification rate decrease of 20% or more compared to the untreated control measurements. At the European Commission drinking water regulation limit concentration of 0.5 μM Cr(VI), the acidification signals need 16–20 h to decrease to 80% of the control (data not shown).

### *4.4. Measurement of respiration rates – Bionas 2500 Analyzing System*

The respiration rate of the cells, measured with a Clark-type electrode, is derived from the oxygen consumption during the stop phase. Cr(III) treatment did not influence the cellular oxygen uptake as the respiration did not change, or only to a minor degree at high concentrations of 50 μM respectively (Fig. 5a). In accordance to the results of the impedance and acidification measurements, it can be stated that the Cr(III) species is not detectable by the cell-based sensor systems.

In contrast to Cr(III), the cytotoxic Cr(VI) is detected in a concentration range between 0.5 and 25 μM within a few hours (Fig. 5b). The exposure to 5 and 25 μM Cr(VI) leads to a sudden breakdown of the cellular respiration, which reaches the absolute limit within 3 h. Even lower concentrations of 0.5 and 2 μM Cr(VI) reach a level below 20% of control after 6–9 h of exposure. The formation of intermediate metabolic products, which influences the cellular respiration chain, is most likely to be the reason for the reduced oxygen consumption rates. Respiration rates indicate the presence of Cr(VI) in a concentration dependent manner, as the slope of the decrease is stronger with increasing amounts. The lowest detection limits from all systems tested in this study were detected via the respiration monitoring (Table 1).

Three impedance sensor systems were investigated within this study. The CMOS chip was tested for the first time with an environmentally relevant toxicant. The initial change of the medium after the cell settlement in the adhesion phase has unfortunately a remarkable impact on the measured impedance values as the frequency rises for several kilohertz. However, combining the frequency measurements with the calculated Vitality Index, the presence of 50 μM Cr(VI) in the solution could be detected within 20 h. Further developments have to be done to increase the performance of the sensor. In contrast, the xCELLigence system as well as the 2500 Analyzing System are well developed devices, both with Cr(VI) detection limits of 5 μM for 20 h exposure. Differences in the signal intensity may occur due to the different coverage of the sensor chip with the interdigitated electrode structure. While the xCELLigence system uses wells with a bottom surface covered to 80% with the electrode structure, the impedance electrode of the Metabolic Chip SC 1000 covers only 12%. The possibility to detect an impedance changing event, which is limited to a small space (e.g. cell death of single cells), is increased with increasing surface coverage by the impedance electrode. For toxicants like Cr(VI), which are supposed to cause homogeneous alterations of all cells on the sensor chip, the influence of the electrode is of a minor extent. With the xCELLigence system, experiments are performed in a batch system. Every medium exchange has to be done manually in the 96-well plate. The 2500 Analyzing System operates in a stop/go-modus via a pumping system which reduces the laboratory work and enables long time studies of effects on the cells. The xCELLigence system is advantageous in the amount of assays which can be performed in parallel (96 wells) compared to the 2500 Analyzing System (6 modules).

Both tested acidification measurement systems (Cytosensor and 2500 Analyzing System) were able to detect changes in the acidification rate of V79 cells during the exposure to Cr(VI) and to resist Cr(III). The Cytosensor has a detection limit after 3 h of about 50 μM Cr(VI) while the 2500 Analyzing System detected concentrations of 5 μM. The increased sensitivity might be partially a result of the fact, that five ISFET-electrodes are used to generate the acidification rate signal. Therefore, the system is more stable toward the malfunction of a single electrode. In addition, the cells are placed in direct contact with the ISFET electrode, while in the LAPS device, the cells are grown on a membrane insert and have to acidify the volume underneath.

The respiration rate monitoring of the 2500 Analyzing System has the highest sensitivity as it is capable of detecting concentrations of 0.5 μM Cr(VI) in water and therefore fulfills the detection limits of the European drinking water regulation. The metabolic intermediates, induced inside the cell by the reduction of Cr(VI) inhibit the cellular respiration system immediately. An overview of the achieved sensitivity results and detection limits of all tested sensor types and devices is given in Table 1. In comparison to a standard cytotoxicity assay (MTT-assay), cell-based sensors showed to be in the same range of sensitivity or even above.

However, the differentiation is based on the different toxic effects and not on the ion species itself. Thus, cell-based sensors as described within this study are only able to distinguish between toxic or non-toxic properties but not between individual toxins. This lack of molecular selectivity has to be kept in mind if the solution an analytical problem, requires the specific detection of one

**Table 1**
Limit of detection of the investigated sensor devices.

| Device | Signal change >10% of control after 6 h | | |
|---|---|---|---|
| | Imp | pH | $O_2$ |
| 2500 Analyzing System (Bionas) | 25 μM | 2 μM | 0.5 μM |
| xCELLigence (Roche) | 25 μM | – | – |
| Cytosensor (Molecular Devices) | – | 50 μM | – |
| CMOS chip (Siemens) | 50 μM | – | – |

single substance but not the overall toxicity (e.g. accumulation of a single heavy metal ion species in environmental probes like ground water). In this case, classical chemical analytical methods like high performance liquid chromatography of spectroscopic methods are indeed still the method of choice.

Finally, one has to take into account, that even these high-profile cell-based sensor systems need relatively long assay times. Bioluminescence or other bioelectricity approaches demonstrated to be able to deliver a faster response toward toxic pollutants [45,46] compared to the above described sensor systems.

## 5. Conclusion

Four mammalian cell-based sensor systems, including the 2500 Analyzing System, xCELLigence system, Cytosensor Microphysiometer and an in house CMOS impedance sensor, have been evaluated for their ability to detect toxic Cr(VI). All devices were able to detect the presence of Cr(VI) in concentrations of at least 50 μM within 6 h of exposure. Monitoring the respiration rates, Cr(VI) could be detected down to a level of international drinking water regulations. In addition, these devices were able to differentiate between the toxic Cr(VI) and the non-toxic Cr(III). The systems were compared by using the same cell line to avoid differences in the sensitivity based on the type of cell line. The study demonstrated that cell-based sensors are powerful tools in the detection of pollutants and the monitoring of contaminations of aquatic systems.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2013.02.105.

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## Biographies

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**Carl Frederik Werner** was born in Göttingen, Germany, in 1982. He received his diploma in 2008 in micro system technology from the University of Applied Sciences Kaiserslautern. He started his Ph.D. thesis in 2008 at the Institute of Nano- and Biotechnologies (Aachen University of Applied Sciences) in collaboration with the Philipps University Marburg. From October to December 2010 he was working under a scholarship from the Marubun Research Promotion Foundation at the Tohoku University in Japan. His research subjects concern chemical and biological sensors, especially the light-addressable potentiometric sensor together with microorganisms and sensor-signal processing.

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**Doris Schmitt-Landsiedel** is head of the Institute of Technical Electronics at Technical University of Munich since 1996. Before, she worked at Siemens on design of analog, digital, memory and circuits for digital hearing aids. She is senior member of IEEE and member of Acatech, the German Academy of Science and Engineering. Her research is in design of reliable mixed signal CMOS circuits, circuits with novel devices and nanomagnetic computing.

**Maximilian Fleischer** heads the Chemical Sensors Research Group of Siemens Corporate Research and Technology. He received the doctoral degree in Physics from the Technical University in Munich in 1992, his habilitation in Physics in 1998 and his honorary professorate in 2009 from the Technical University of Budapest. His work includes piezoelectric motors, new types of semiconducting metal oxides, high-temperature electrochemical sensors, tunable laser diode spectroscopy for gas sensing, NIR-spectroscopy for on-line analysis of condensed media, low-power work function based gas sensors and living cell-based sensors.

**Patrick Wagner** obtained his Ph.D. in 1994 at Technical University Darmstadt (Germany) in experimental solid state physics with a focus on cuprate superconductors. From 1995 until 2001, he was postdoctoral researcher in the Laboratory of Solid State Physics and Magnetism at Catholic University Leuven (Belgium), where he studied the magneto-transport properties of mixed-valency magnetic oxides. Since 2001, he is a professor of physics at Hasselt University (Belgium) and responsible for the development of label-free readout techniques for DNA-, protein-, and small-molecule sensors. Patrick Wagner is recipient of a WE Heraeus award, a Marie-Curie Fellowship of the European Union, a Methusalem Grant of the Flemish Government and is past president of the Belgian Physical Society.

**Michael J. Schöning** received his diploma degree in electrical engineering (1989) and his Ph.D. in the field of semiconductor-based microsensors for the detection of ions in liquids (1993), both from the Karlsruhe University of Technology. In 1989, he jointed the Institute of Radiochemistry at the Research Center Karlsruhe. Since 1993, he has been with the Institute of Thin Films and Interfaces (now, Institute of Bio and Nanosystems) at the Research Center Jülich, and since 1999 he was appointed as full Professor at Aachen University of Applied Sciences, Campus Jülich. Since 2006, he serves as a director of the Institute of Nano- and Biotechnologies (INB) at the Aachen University of Applied Sciences. His main research subjects concern silicon-based chemical and biological sensors, thin-film technologies, solid-state physics, microsystem and nano(bio-)technology.